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OXYGEN UPTAKE AND TRANSPORT IN THE PROSOBRANCH MOLLUSC *BUSYCON CANALICULATUM*. I. GAS EXCHANGE AND THE RESPONSE TO HYPOXIA[1]

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The oxygen equilibrium properties of gastropod hemocyanins have been studied *in vitro* since the early work of Redfield, Coolidge and Hurd (1926) and Redfield and Ingalls (1933). These workers showed that oxygen binding of the blood of the prosobranch conch *Busycon canaliculatum* (L.) is cooperative and that the pH dependence of oxygen affinity is reversed relative to that of mammalian hemoglobins. It is now known that oxygen affinity at 22° C decreases from very high to moderately low in the pH range 6.6 to 7.9, above which the large reverse Bohr shift is abruptly replaced by a small normal Bohr shift (Mangum and Lykkeboe, 1979). The physiological meaning of this complex relationship is not clear, however, because the respiratory variables in the blood are largely unknown. The other example of a reverse Bohr shift in a potentially physiological pH range occurs in the xiphosuran arthropods, where the higher oxygen affinity at low blood pH clearly enhances oxygen uptake in hypoxic water (Johansen and Petersen, 1975; Mangum, Booth, deFur, Heckel, Henry, Oglesby and Polites, 1976). Unlike the horseshoe crabs, however, the shelled molluscs have, in direct contact with the tissues, a massive reservoir of $CaCO_3$ that may dissolve and prevent appreciable changes in blood pH (Mangum, Henry and Simpson, 1979).

Quite apart from the response to hypoxia, the respiratory role of gastropod hemocyanin under normoxic conditions cannot be inferred from the information available on other classes in this heterogeneous phylum. Recent studies of the blood oxygen carriers in species representing three of the seven molluscan classes suggest a diversity not found in many animal phyla. In the Amphineura (Redmond, 1962; Petersen and Johansen, 1973) and the nautiloid Cephalopoda (Johansen, Redmond and Bourne, 1978), a relatively small fraction of the oxygen available in the blood is actually delivered to the tissues, and the venous reserve is conspicuously large; this relationship suggests that the blood may be more highly adapted to environmental or behavioral conditions that have not yet been investigated. In the hemoglobin-containing Lamellibranchia, the blood delivers about half of its oxygen load and thus supplies two-thirds of total oxidative metabolism, a common pattern among the various animal phyla (Deaton and Mangum, 1976; Freadman and Mangum, 1976). In contrast, the respiratory role of the blood in either octopod (Johansen, 1965; Johansen and Lenfant, 1966) or decapod (Redfield and Goodkind, 1929) Cephalopoda is truly outstanding in the animal kingdom; very little oxygen remains when the blood leaves the tissues.

We have investigated oxygen uptake and transport in both normoxic and hypoxic water in a member of a fourth molluscan class, the gastropod *Busycon canaliculatum* (L.). The species is found in the lower intertidal and upper subtidal (< 30 m) zones from Cape Cod to Florida, where it reaches maximum

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abundance on muddy sediments and thus does not overlap with large numbers of its congener *B. carica*.

Materials and Methods

Living material

Specimens of *Busycon canaliculatum* (L.) were obtained from the Chesapeake Bay and various inlets of the Atlantic coast of Virginia. A few observations were made on *Murex fulvescens* Sowerby, from waters near Beaufort, North Carolina, and on *Lunatia heros* (Say), from Woods Hole. Animals were maintained without feeding in natural sea water at 31 to 35‰ salinity and 21 to 24° C. Most of the measurements were made in large aquaria of recirculating sea water, but it was necessary to conduct some of the experiments in the running sea water system of the Wachapreague Laboratory of the Virginia Institute of Marine Science. The animals were always held under the experimental conditions specified for 5 to 10 days prior to a measurement.

Oxygen uptake

Several days before an experiment, the shells were scrubbed and then dipped quickly into melted paraffin wax, followed by iced sea water, to eliminate oxygen uptake by epibiota; as reported previously, this procedure does not cause mortality (Kushins and Mangum, 1971). The animals were placed in a vessel, which was either aerated or flushed with running sea water for several hours until the siphon formed and ventilation resumed. Then the vessel was sealed and the depletion of oxygen monitored with a Yellow Springs Instruments 5420A electrode. Indicator dyes showed that mixing with the self-stirring device was adequate in the smaller ($<$ 500-ml) vessels, but supplementary stirring was provided at the bottom of larger vessels.

The oxygen uptake of various excised tissues was measured with a Gilson differential respirometer, and with a Yellow Springs Instruments Model 53 Cell Respiration apparatus.

Oxygen extraction and ventilation

Animals were suspended and held stationary in the water column in an aquarium of standing water, to allow continuous measurements. P_{O_2} of the excurrent was measured with a Beckman hypodermic microelectrode and Model 160 Physiological Gas Analyzer; P_{O_2} of the incurrent was noted at the same time with the YSI macroelectrode. Ambient P_{O_2} was lowered with N_2 in steps at the rate of about 20 to 30 mm Hg/hr. At each P_{O_2}, several measurements were made for 1 to 5 min each, over a total period of at least 30 min, estimated from the area under a continuous trace.

Water flow was estimated by two methods. First, a thermistor flow probe (LaBarbera and Vogel, 1976) was lowered into the excurrent generated by conchs suspended in a darkened aquarium of standing water, as above. Attempts to record flow into the siphon were unsuccessful because its margins move continuously, touching the probe and inducing the withdrawal response, and also changing both the number and the size of incurrent openings. The records of the excurrent, which is more diffuse but also more constant in shape, were cali-

brated volumetrically with a Gilson Instruments manometric flowmeter attached to a plastic funnel, made to approximate the dimensions of the exhalant opening. Second, volumetric flow was estimated by attaching the manometer directly to the siphonal canal of the shell. In running sea water, four freely locomoting conchs extended the siphon into the connector tube so fully that a watertight junction was formed. However, they would not ventilate against the resistance of this apparatus unless an open tube was placed in parallel with the manometer: after disconnecting it from the shell, the readings from this Y-shaped apparatus were calibrated with a second manometer.

Blood gases and pH

Many unpaired samples of blood were obtained quickly (< 10 sec) and easily by hypodermic syringe sampling of the large cephalopedal sinus of freely locomoting animals, without apparent harm to the conch. Paired samples of blood were obtained from the afferent and efferent sinuses at the margin of the gill, after inserting the claw of a hammer into the grooves of the shell coils and quickly removing two large pieces before appreciable retraction could occur. Our joint participation was required to complete this operation within 10 to 20 sec of initial contact. Additional, unpaired samples were obtained quickly (< 10 sec) from the ventricles and nephridia of different animals.

Blood gases and pH were measured with a Radiometer BMS1 blood gas analyzer. After air equilibration, oxygen carrying capacity was measured with a Lexington Instruments (Lex-O_2-Con-TL) oxygen concentration analyzer. Because the results seemed anomalous, hemocyanin concentration was estimated from absorbance at 345 nm (Beckman DK-2 spectrophotometer), using the extinction coefficients given by Nickerson and van Holde (1971), and oxygen concentration predicted from Cu content and Cu: O_2 combining ratio (Van Bruggen and Van Holde, 1971).

The blood was equilibrated *in vitro* to various mixtures of N_2, O_2 and CO_2, prepared with Wösthoff pumps. Then its pH was measured with a Radiometer BMS3 pH electrode.

Results

Behavior and mode of gas exchange

In tanks of rapidly running sea water, *B. canaliculatum* often climbs to the surface and extends its siphonal canal into the air. In a more natural posture, however, we were unable to detect any indication of air-breathing. When crawling on sand in an aquarium, conchs responded to a slow lowering of the water level by retracting into the shell. During four days of observing animals in nature at low tide, we invariably found *B. canaliculatum* (30–35 individuals) burrowed partly into the mud so that the siphonal canal was covered. We conclude that, in nature, the mode of gas exchange is exclusively aquatic.

Submerged animals crawl along the bottom, rotating the shell in wide arcs alternately to the left and right. At irregular intervals the pedal muscles contract, elevating the siphonal canal and increasing the volume of the mantle cavity. This motion thoroughly mixes the water in the mantle cavity, possibly enhancing the uptake of oxygen at the mantle and gill. When this response occurred during the measurements of flow and oxygen extraction, the data were discarded.

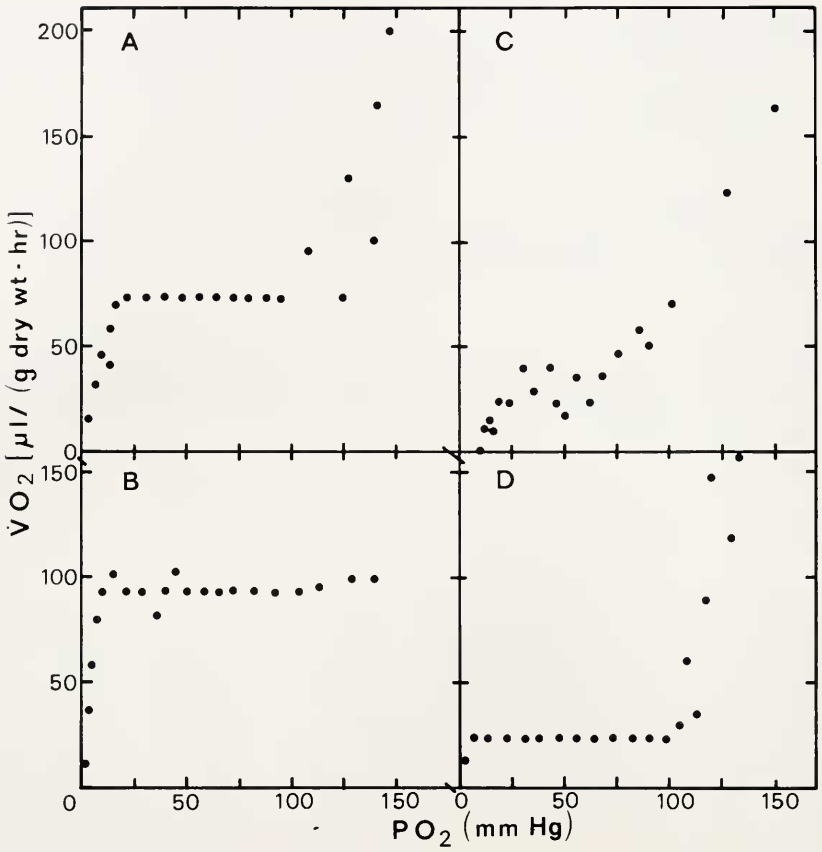

FIGURE 1. The relationship between oxygen uptake ($\dot{V}_{O_2}$) and ambient P_{O_2} in large marine prosobranchs. A. *Busycon canaliculatum* [24-gm(dry wt) animal]. B. Isolated pedal muscle fibers from a specimen of *B. canaliculatum*. C. *Lunatia heros* [5.4-g(dry wt) animal]. D. *Murex fulvescens* [20-g(dry wt) animal] 22 to 23° C, 31 to 32‰ salinity.

Oxygen uptake

The relationship between oxygen uptake and ambient P_{O_2} in *B. canaliculatum*, which is unusually complex, appears to be characteristic of large prosobranch gastropods in warm water (Fig. 1). Essentially there are three distinct components: First, above 120 mm Hg, oxygen uptake changes exponentially with ambient P_{O_2}. The animal usually keeps most of its body inside the shell and does not locomote rapidly, although the siphon is open. The response to mechanical stimulation is conspicuously slow. Secondly, between 40 to 50 and 100 to 120 mm Hg, a range often observed in nature (Deaton and Mangum, 1976), the rate of oxygen uptake is strongly regulated, and behavioral responses to external stimuli are far more rapid. Thirdly, in 17 of 23 animals tested, the rate of oxygen uptake declines below 40 to 50 mm Hg (*e.g.* Fig. 1A).

This complex relationship is found even when the animal is allowed to adjust to the container in running sea water for 24 hr prior to initiating the measurement, and it recurs in as many as three sequential measurements made in tandem. Although no observations of behavior were made, the same pattern was found

TABLE I

Oxygen uptake ($\dot{V}_{O_2}$) of tissue isolated from Busycon canaliculatum. *35‰, 22° C. mean ± s.e.*

Tissue	$\dot{V}_{O_2}$[(μl/(g dry wt·hr)]	N
Pedal muscle	111 ± 4	9
Cardiac muscle	436 ± 17	20
Pedal epidermis	271 ± 14	13
Gill	794 ± 16	17
Nephridium	318 ± 33	17

in two other large gastropods (Fig. 1C, D). In both of the hemocyanin-containing species, *B. canaliculatum* and *M. fulvescens,* oxygen uptake always persists until no oxygen can be detected in the medium, but in *L. heros,* which lacks a blood oxygen carrier, oxygen uptake becomes imperceptible below 10 mm Hg. Despite the production of lactate during anaerobiosis in gastropods (Ellington, Long and Duda, 1977), there is no clear evidence of an increase in oxygen uptake following brief exposure to hypoxia during the second or third of the sequential measurements.

At 21 to 23° C, 31 to 34‰ salinity and ambient $P_{O_2} = 100$ to 120 mm Hg, the mean value for oxygen uptake in 12 animals of intermediate body size (22–27 g dry wt) is 82 (± 4 s.e.) μl/(g dry wt·hr). The maximum rate at $P_{O_2} > 140$ mm Hg, which is far more variable, is 200 (± 23 s.e.) μl/(g dry wt·hr). The weight-specific rate of oxygen uptake by most of the isolated tissues in air saturated water exceeds that of the animal as a whole (Table I, Fig. 1) by a large margin. This relationship could be explained alternatively as follows. Either, $\dot{V}_{O_2}$ (whole animal) is an average of the values in Table I, weighted according to the relative mass of each tissue. This explanation requires the condition that the pedal muscle comprises more than 2/3 of total body weight, which is untrue. Or, $\dot{V}_{O_2}$ (whole animal) is an average of the values for the various tissues, weighted according to their biomass and also according to their ambient (*viz.* blood) P_{O_2}. This explanation, which is far more consistent with tissue weight distribution, requires the condition that the oxidative demand of most of the tissues in an animal is not fully satisfied by the supply. This hypothesis is strongly supported by measurements of oxygen uptake in pedal muscle fibers (Fig. 1); in none of the 4 experiments was the rate maintained below 10 mm Hg.

Ventilation

The water current enters the mantle cavity through the siphon, a tube formed transiently by the mantle. The current enters the mantle cavity and then passes obliquely over the head and gills. After flowing over the excretory openings, the water exits the shell from the side of the mantle cavity opposite to the siphon, as a diffuse current. Extensive mixing in the excurrent is often observed when particles are present in the water.

In addition to ventilating the two sites of oxygen uptake into the blood, the current flushes poorly vascularized epithelium. Oxygen is also taken up from water that is not actively moved by the animal, at the leading and trailing surfaces of the foot, and the rate of oxygen uptake by these epithelia is relatively high (Table I). Thus, not all of oxidative metabolism is fueled by the diffusion of oxygen from the actively generated water current into the blood.

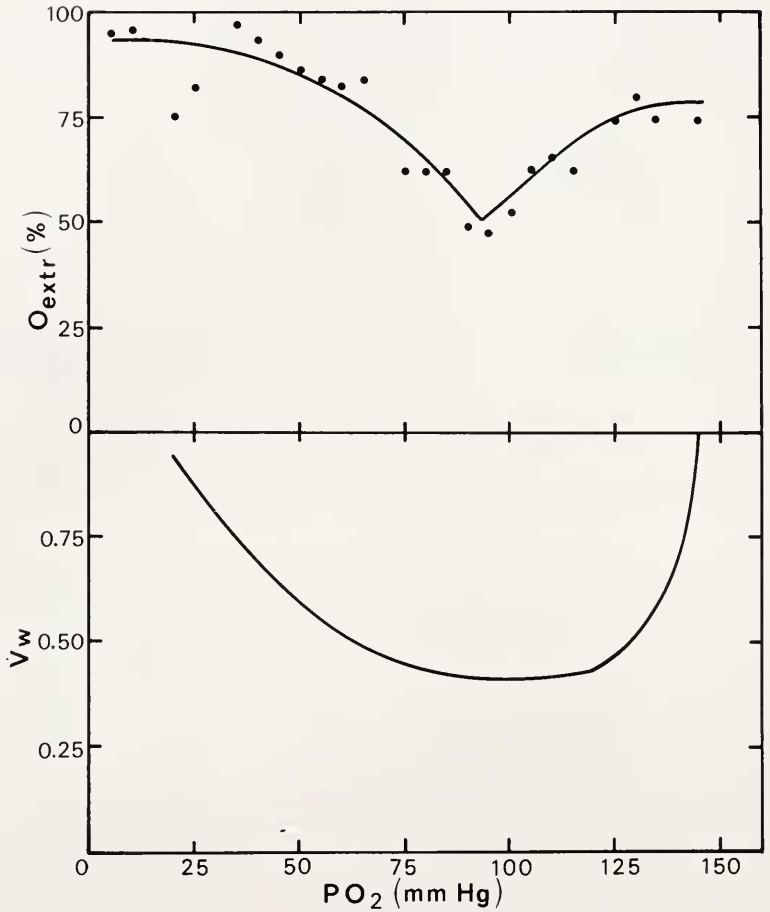

FIGURE 2. The relationship between oxygen extraction (O_{extr}, top), ventilation (V_w, bottom) and ambient P_{O_2} in *Busycon canaliculatum* (22–24° C, 32–34‰ salinity).

The flow rate varies considerably over short periods, which might lead to appreciable errors in estimating ventilation from point observations with a manometer. On the other hand the calibration of the records made from the diffuse excurrent requires precise simulation of the cross-sectional area of the current, which is difficult. In addition, the posture of the animals was not the same during the two sets of measurements. Nonetheless, the agreement between the data obtained with the two methods is quite good, and we suggest that they are reasonably accurate. The most stable value obtained with the manometer is 15 ml/(g dry wt·hr), and the value estimated from the integral of the continuous record is 14.3 (± 1.1 s.e.) ml/(g dry wt·hr) ($N = 9$). The rate predicted from the data for total V_{O_2} and per cent O_2 extraction is 18.3 ml/(g dry wt·hr). The discrepancy may be due to an assumption involved in making this calculation, *viz.* that all of oxygen uptake is fueled by active ventilation, which is incorrect. The difference of about 15 to 20% may represent the passively ventilated component of oxygen uptake.

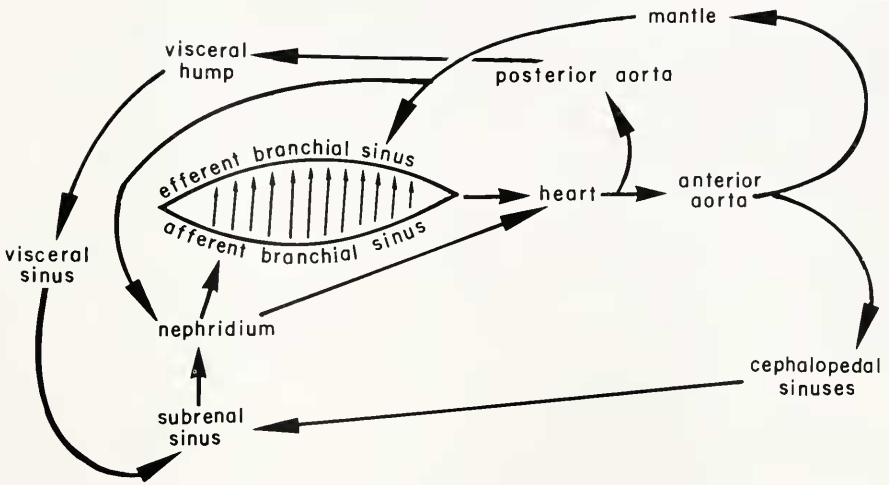

FIGURE 3. The path of blood flow in *Busycon canaliculatum*.

Oxygen extraction

The complexity of the relationship between per cent oxygen extraction and ambient P_{O_2} parallels that between $\dot{V}_{O_2}$ and P_{O_2} (Fig. 2). The two relationships are, of course, interdependent. In the range 100 to 159 mm Hg, both O_2 uptake and O_2 extraction change exponentially. At 100 mm Hg, O_2 extraction reaches the level expected in an animal that generates a respiratory and not a trophic water current (Mangum, 1977). At 25 to 100 mm Hg, oxygen extraction steadily increases, thus permitting the regulation of oxygen uptake in spite of the decreasing oxygen supply in the medium.

No measurements of water flow were obtained at low P_{O_2}, due to behavioral responses that interfered with each of the two methods. The relationship between $\dot{V}_w$ and ambient P_{O_2} can be predicted, however, from the data for $\dot{V}_{O_2}$ and per cent O_2 extraction, assuming no effect of P_{O_2} on the partitioning of oxygen uptake between actively and passively ventilated gas exchange sites. This relationship is shown in Figure 2 in arbitrary units, because the absolute values would be somewhat high (see above).

Circulation of the blood

The pattern of blood flow is diagrammed in Figure 3, which is based on the accounts of the anatomy of the system by Dakin (1912) and Pierce (1950), and a few personal observations. Blood emerges from the heart into an aorta which immediately divides into two branches. The smaller of the two, the posterior or ventral aorta, supplies the deep visceral tissue, intestine and gonad, that fills the innermost coils of the shell. Blood returning from these tissues collects into a perivisceral sinus and then passes to the subrenal sinus and the nephridium. Blood leaving the nephridium may pass into the gill or directly into the auricle as in other molluscs. The much larger anterior or cephalopedal aorta gives off a pallial branch and then proceeds along the esophagus, where its additional branches supply the feeding and digestive organs, and into the foot and head regions, where it enlarges to form a secondary pump. The blood leaving the digestive, sensory

TABLE II

Respiratory properties of the blood of Busycon canaliculatum. *22 to 24° C, 32 to 34‰ salinity, ambient P_{O_2} 100 to 120 mm Hg, ambient P_{CO_2} 1.0 mm Hg. mean ± s.e. (N).*

Blood pH	
postbranchial	8.04 ± 0.09 (4)
pedal	7.86 ± 0.01 (23)
prebranchial	7.85 ± 0.01 (4)
Blood P_{O_2} (mm Hg)	
postbranchial	27 ± 7 (9)
cardiac	21 (2)
pedal	5.5 ± 1.3 (10)
renal	5.3 (2)
prebranchial	2.3 ± 0.4 (11)
Blood P_{CO_2} (mm Hg)	
postbranchial	1.1 (2)
prebranchial	1.8 (2)
Blood ammonia (mM/liter)	
postbranchial	0.03 ± 0.01 (6)
pedal	0.08 ± 0.02 (18)
prebranchial	0.09 ± 0.01 (6)
Blood O_2 carrying capacity	3.68 ± 0.29 (12)

and pedal structures collects in the large cephalopedal sinuses, and passes from there into the subrenal sinus, through the nephridium and then either to the gill or heart. Thus the blood travelling this route, which appears to be diluted with sea water in the foot (Mangum, 1979), may return to the tissues without being oxygenated, but not without being filtered. Although Pierce (1950) makes no mention of the pallial branch of the anterior aorta, a third major route of circulation, it is clearly visible in *Busycon* as a large vessel that extends into superficial epithelium of the mantle. If its position in the system is the same as in the related neptuneid conch *Buccinum* (Dakin, 1912), it branches off before the anterior aorta reaches the esophagus and extends throughout the dorsal part of the mantle and the siphon. Blood returning from the mantle, a site of oxygen uptake, enters either the heart or the efferent branchial sinus, and thus mixes with the blood leaving the gill, a second site of oxygen uptake. According to Dakin (1912), some of the blood travelling the pallial route is also filtered by the nephridium before returning to the heart.

Blood pH and buffering

As the blood flows through the circulatory system, the pH change is exceptionally large for an aquatic animal (Table II). The largest change appears to occur between the gill and the pedal sinus; there is little further net increase in acidity as the blood is filtered and returned to the gill.

The reverse Bohr shift contributes to the large pH change (Fig. 4); unlike bloods containing carriers with a normal Bohr shift, deoxygenation results in an increment in acidity. However, the effect of oxygenation state on pH is small. Similarly, blood ammonia levels are always low, as is the rate of ammonia excretion (Polites and Mangum, 1979). In contrast, the change in total CO_2 as the blood travels through the system (> 0.04 mM) is more than sufficient to explain the

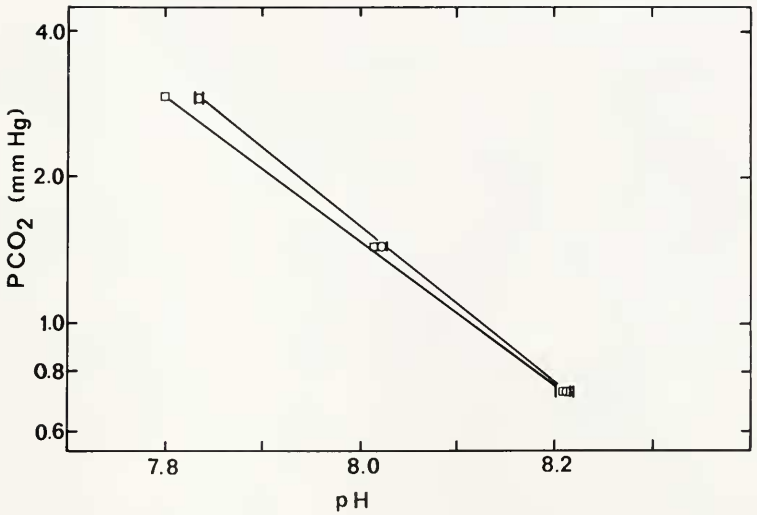

FIGURE 4. Buffer properties of oxygenated (open circles) and deoxygenated (open squares) blood taken from *Busycon canaliculatum* (22° C, 31‰ salinity).

observed change in pH. Whether the relatively large CO_2 change is due to a slow rate of blood flow or, equally likely, to the special CO_2 requirements of shell secretion, is not clear, but we suggest that the primary agent of the pH change is the exchange of a component of the CO_2 system.

When animals are held in hypoxic water ($P_{O_2} < 20$ mm Hg) for 6 hr, pedal blood pH (7.82 ± 0.03 s.e.) and P_{CO_2} [1.5 (± 0.2 s.e.) mm Hg] change very little (N = 6). Moreover, there is no increase in free Ca^{+2} content of the blood (Mangum and Lykkeboe, 1979), suggesting that the shell is not an important source of buffer under these conditions. When exposed to hypoxia for longer periods (12–24 hr), pedal blood pH dropped to 7.4 to 7.6. However, none of six animals in each of two experiments survived this experience, and no further measurements were attempted.

Oxygen carrying capacity

Numerous samples of blood from the pedal sinus of freely locomoting animals yielded values for oxygen carrying capacity that were unexpectedly low [1.19 (± 0.06 s.e.) ml/100 ml, N = 58]. They were, however, consistent with predictions based on hemocyanin concentration [1.39 (± 0.07 s.e.) ml/100 ml, N = 11]. Surprisingly, the values for samples taken from the nephridium of the same animals were 2 to 4 times higher. This apparent anomaly occasioned a more intensive study of the changes in hemocyanin levels in different parts of the circulatory system and in the water extruded from the shell as the animal withdraws, which was undertaken in the closely related species *Busycon carcia* because of its greater abundance (Mangum, 1979). Although fewer data are available for *B. canaliculatum* (Table II), they are entirely consistent with the findings for *B. carcia:* when the animal inflates its foot, pedal blood appears to be diluted, presumably with sea water, and the blood is reconcentrated before returning to the sites of oxygen uptake. The figures for oxygen carrying capacity given in Table II represent branchial, cardiac and nephridial blood.

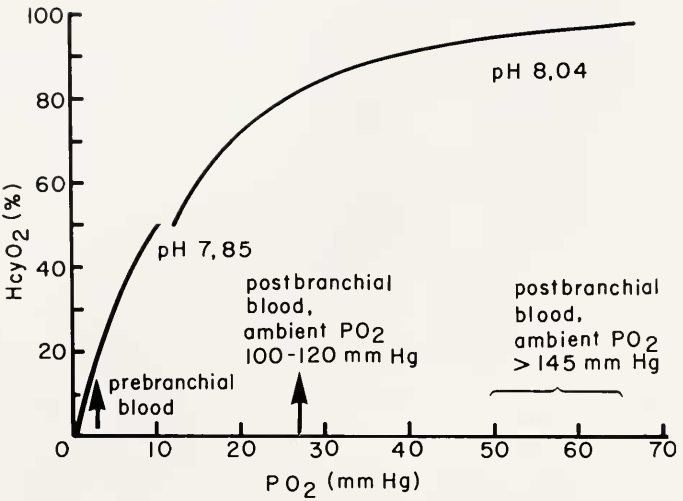

FIGURE 5. Oxygen equilibrium of the blood taken from *Busycon canaliculatum* at postbranchial and prebranchial blood *p*H (22° C, 31‰ salinity). From data reported by Mangum and Lykkeboe (1979). Brackets show range of blood P_{O_2} when ambient water $P_{O_2} = 145$ to 159 mm Hg.

Blood P_{O_2} and oxygenation

When animals are held at an ambient P_{O_2} of 100 mm Hg, where the rate of oxygen uptake is stable, blood P_{O_2} at the efferent margin of the gill usually falls within the range 20 to 30 mm Hg. At ambient $P_{O_2} = 145$ to 159 mm Hg, the values are far more variable, although they fall in the range 50 to 65 mm Hg when the siphon is clearly protruded. As expected from the mass of tissue supplied with oxygen at a particular site in the circulatory system, the largest decrease in blood P_{O_2} occurs in the pedal sinus (Table II), and the further change is relatively small. Prebranchial blood P_{O_2} varies very little, regardless of the oxygen level in the medium.

In air-saturated water, the hemocyanin in the blood is essentially oxygenated in full at the gill (Fig. 5), and at 100 to 120 mm Hg the per cent oxygenation is almost as great. Although most of the O_2 load is delivered before the blood leaves the pedal sinus for the nephridium, the change in oxygenation accompanying the small drop in P_{O_2} between the foot and the gill results in the delivery of about 0.3 ml O_2/100 ml blood, which is a substantial supply for the nephridium, a relatively small mass of tissue. The venous reserve of O_2, or the amount remaining in the blood as it returns from the tissues, is exceptionally small. In view of the low estimate of blood flow (Table III), which implies a long equilibration time at the tissues, and also the locomotor capability of the animal, which was essentially maximal under the conditions studied, this finding is not surprising.

DISCUSSION

In comparison with the other molluscs studied, the rate of total oxidative metabolism is higher than expected in *Busycon canaliculatum*, especially considering its low level of motor activity in relation to many other molluscs (Table III). Comparing the regulated rates, which we suggest are more meaningful, *B. canaliculatum* has a higher capacity for oxidative metabolism (2–3 times) than the

TABLE III

Respiratory variables in six species of molluscs. Data for Octopus, Nautilus *and* Cryptochiton *converted by assuming that dry wt = 20% wet wt. Ambient* P_{O_2} *(*PiO_2*) > 140 mm Hg unless specified otherwise.*

	Busycon canaliculatum	*Cryptochiton stelleri*	*Noetia ponderosa*	*Modiolus demissus*	*Nautilus pompilius*	*Octopus dofleini*
Body wt., dry (g)	24	*ca.* 80	5	1.35	*ca.* 116	*ca.* 2020
Experimental temperature (°C)	21–23	10	21–23	21–23	16–20	8–11
$\dot{V}_w$ [ml/(kg dry wt·min)]	238–250	*ca.* 380	267	2233	*ca.* 1480	*ca.* 1360
O_2 extraction from water (%)	50*	48	60	8–9	7	80
$\dot{V}_{O_2}$ [ml/(kg·min)]	1.17*	*ca.* 0.33	5.27	7.88	*ca.* 2.25	*ca.* 1.97
Blood O_2 carrier	Hcy	Hcy	Hb	none	Hcy	Hcy
% oxygenation						
postbranchial	84*	93	99		99	94
prebranchial	16*	74	52		63	21
Blood O_2 delivered to tissues (ml/100 ml)	2.25*	0.36	1.82	0.06	1.1	2.9
O_2 extraction from blood (%)	84	48	47	14	*ca.* 40	80
Cardiac output [(ml/(kg·min)						
Fick principle	52*	92	125	1313	205	68
heart rate × volume	26*			1241		
Source	present data, deFur and Mangum (1979); Mangum and Lykkeboe (1979)	Redmond (1962) Petersen and Johansen (1973)	Deaton and Mangum (1976), Freadman and Mangum (1976)	Booth and Mangum (1978)	Redmond, Bourne and Johansen (1978), Johansen, Redmond and Bourne (1978)	Johansen (1965), Johansen and Lenfant (1966)

* PiO_2 = 100 to 120 mm Hg.

other large prosobranchs examined, including the hemocyanin-containing *Murex fulvescens* (Fig. 1). Members of the genus *Murex* have an appreciably lower rate (38%) of oxygen extraction as well (Hazelhoff, 1939).

The high capacity for oxidative metabolism does not result from a high rate of oxygen convection on either side of the respiratory epithelium (Table III). Oxygen extraction, both from ambient water and from blood, is quite great, which emphasizes the importance of the relatively long equilibration periods accompanying low rates of fluid flow, in spite of the smaller P_{O_2} gradient driving oxygen into the tissues. The oxygen-carrying capacity of *Busycon* blood is also relatively high, although none of the bloods with hemocyanin reach the capacity of the hemoglobin-containing blood of the ark clam *Noetia*. We suggest that oxygen uptake in *B. canaliculatum* is also enhanced by the more or less serial arrangement of the two sites of oxygen uptake, the mantle and the gill, which greatly extends the distance over which oxygen can be extracted from the water current. Positioned first in the series where they extract the most highly oxygenated water are the smaller pallial vessels and then the gill, which essentially act as preamplifiers of blood oxygen. Next in the series are the larger pallial vessels, which discharge into the efferent branchial sinus. The importance of this arrangement can be inferred from the data for normoxic water. At an incurrent water P_{O_2} of about 130 to 159 mm Hg, oxygen extraction is 75%; the P_{O_2} of the excurrent is 33 to 40 mm Hg. And yet postbranchial blood P_{O_2} reaches levels of 50 to 65 mm Hg. The inverse relationship between water and blood P_{O_2} can be possible only if the blood at the first site of oxygen uptake is considerably more oxygenated than the blood at the second site in the series, and/or if a very large fraction of additional oxygen is removed after the current passes the two sites of oxygen entry into the blood.

The mantle is exquisitely sensitive to mechanical stimulation, and we were unable to estimate its gas exchange surface in an animal assuming its ventilation posture. However, we must emphasize that the figure of 8 cm^2 gill surface area/gm wet wt (Ghiretti, 1966), only 10 to 15% lower than in other molluscs with a non-trophic gill, vastly underestimates the total surface available for gas exchange in neptuneid conchs.

The strong regulation of oxygen uptake in the ambient P_{O_2} range 40 to 50 to 100 to 120 mm Hg is due in part to the equally strong regulation of heart rate (de Fur and Mangum, 1979). Unless the partitioning of oxygen uptake between actively and passively ventilated sites changes with ambient P_{O_2}, however, regulation is also due to the increased ventilation predicted by the data for oxygen extraction and oxygen uptake. Such a pronounced response by a ciliary pump is somewhat unexpected, both on *a priori* grounds and on the basis of direct measurements of water flow; in lamellibranchs water flow increases only slightly at intermediate oxygen levels and then sharply falls in hypoxic water (Booth and Mangum, 1978). We cannot dismiss the possibility that our assumption of an unchanged partitioning of oxygen uptake is incorrect. Alternatively, the flow rate may be regulated by a variable under direct control by the central nervous system, such as the geometry of entrance conditions, or the shape of the siphon.

In Table III cardiac output was calculated according to the Fick principle, using the data for total oxygen uptake and the change in oxygen concentration as the blood passes the tissues. The validity of this calculation requires a condition which is clearly untrue, *viz.* that all of the oxygen consumed is first taken up by the blood. Not surprisingly, in view of the large surface area of unvascularized tissue exposed directly to the ambient medium, the result is considerably higher than the figure obtained by an alternative method. DeFur and Mangum (1979) calculated cardiac output from measurements of heartrate times stroke volume, which was deliberately estimated on the conservative side. Although the alternative figure may be somewhat low, it is unlikely to be in error by as much as 50%, and the discrepancy is far more likely to be real, due to the uptake of oxygen by unvascularized epithelium, the rate of which is relatively high (Table I).

The adaptive significance of the reverse Bohr shift in *B. canaliculatum* differs from that in *Limulus* (Johansen and Petersen, 1975; Mangum *et al.*, 1976). Unless the effect of hypoxia on branchial blood pH is fundamentally different from the response of foot blood pH, which does not change significantly until the animal is moribund, then the reverse Bohr shift does not facilitate oxygen uptake in hypoxic water, as it does in *Limulus*. Indeed, oxygen uptake declines sharply in hypoxic water (Fig. 1A). In normoxic water, the change in blood pH at the gill induces a normal Bohr shift which is too small to be physiologically significant. The important point is that the reverse Bohr shift is not the physiological problem often supposed in the past, because blood pH falls below 7.9, the critical value at which the reverse Bohr shift abruptly appears, only slightly and only after the blood has delivered three-quarters of its oxygen load. As suggested earlier (Mangum and Lykkeboe, 1979), the reverse Bohr shift may even be an advantage at this point, because it opposes further depletion of the already small oxygen load in the blood, which must still supply the nephridium before returning to an oxygenation site.

There may be another effect of the decrease of blood pH below 7.9 which is more important than the respiratory effect. The abrupt change from normal to reverse Bohr shift is apparently due to the aggregation of 4.5×10^6 dalton par-

ticles to 9×10^6 dalton particles (DePhillips, Nickerson and Van Holde, 1970). The aggregation state of *B. canaliculatum* hemocyanin is also influenced by deoxygenation, which increases the number of 9×10^6 dalton particles formed. While the rate of aggregation of half particles at pH 8.3 and in artificial media is very slow (> 6.5 hr), the kinetics of disaggregation and of the pH effect are not known. It is possible that the aggregation state changes *in vivo* as the blood flows through the system, which would also result in appreciable changes in the activity of the inorganic ions in the blood (Mangum, unpublished data). Fluid balance between the tissues and either the blood or ambient sea water could be altered by one or both of two mechanisms: the doubling of the colloid osmotic pressure of the hemocyanin particles *per se* and the colligative behavior of the inorganic osmolytes bound or released. These two properties of the blood are presently under investigation by the authors.

The data in Figure 4 were collected by G. Lykkeboe, in collaboration with the authors.

Summary

1. The blood of the conch *Busycon canaliculatum* is highly oxygenated at the two sites of gas exchange with the ambient medium, the gill and the mantle. An exceptionally small venous reserve is maintained in prebranchial blood, suggesting an important respiratory role of the oxygen transport system.

2. In normoxic water the physiological pH range is 7.85 to 8.04, where the reverse Bohr shift of *Busycon* hemocyanin is essentially absent. During non-lethal exposure to hypoxia, blood pH does not decrease substantially and thus the reverse Bohr shift cannot be interpreted as an adaptation to low oxygen conditions.

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